EFFECT OF WATER ACTIVITY ON THE GROWTH OF *STAPHYLOCOCCUS AUREUS* AT MEAT-CHEESE INTERFACES

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ABSTRACT

There is increased marketing of ready-to-eat nonrefrigerated snack foods which consist of meat or sausage products with low or intermediate moisture levels combined with high moisture food products, i.e., cheese products. Packaging the intermediate moisture meat in direct contact with a high moisture food might change the water activity (a_w) of the products sufficiently to support growth of Staphylococcus aureus *at contaminated interfaces. To evaluate this possibility, sterile sausage slices (a_w = 0.60 to 0.82) were surface inoculated with log 2-3 CFU/g of* S. aureus, *interfaced with processed cheese slices (a_w = 0.94), vacuum packaged, and incubated at 19, 28, 37C and at cyclic temperature of 19-37-19C.* S. aureus *levels and water activities were determined weekly for 0 to 9 weeks. The a_w at the interface changed rapidly and reached an a_w that supported* S. aureus *growth. Growth of* S. aureus *occurred under all test conditions when the samples were stored at 28 and 37C. At 19C storage* S. aureus *remained viable for the length of the study.*

INTRODUCTION

Multi-component ready-to-eat snack or refrigerated package foods have increased in the American market place. Within this category, snack foods containing sausage and cheese products have become increasingly popular. The

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production of these nontraditional foods containing components with different water activities (a_w) relies on innovative manufacturing and vacuum packaging technologies. Commercial meat sausage can have an a_w range of 0.73 to 0.90 (Lee *et al.* 1981; Palumbo *et al.* 1979; Tatini 1973) and is considered shelf stable. Commercial processed cheese has an a_w of > 0.94 (Kreisman and Labuza 1978).

Staphylococcus aureus is a particular concern when intermediate moisture meat or sausage is combined with a high a_w product because of the microorganism's ability to grow at a_w levels > 0.83 and its common occurrence in intermediate moisture foods (Lee *et al.* 1981; Kotzekidou 1992). Further, processed cheese products, having a pH range of 5.67 to 5.76 and a_w range of 0.90 to 0.94, support the aerobic growth of this organism (Kreisman and Labuza 1978). Previous reports indicated that *S. aureus* grows in sausages having a pH adjusted to 5.2 (Troller 1986) and survives in an intermediate moisture meat product with a pH of 5.5 (Kotzekidou 1992). The ability of *S. aureus* to grow at reduced a_w levels also depends on storage temperature (Smith *et al.* 1983).

The purpose of this study was to determine the growth potential of *S. aureus* when it is inoculated at the interface of a meat with a low water activity and a cheese with a higher water activity at temperatures of incubation of 19, 28 and 37C and fluctuating temperatures (transitory abuse conditions) of 19-37-19C.

METHOD AND MATERIALS

Meat Sample Preparation

Commercially prepared cooked salami (sausage-10.5 $\pm$ .5 cm diameter) was obtained from a local supermarket and sliced (0.2 cm thick). The total aerobic count of the meat slices before and after drying were enumerated on tryptic soy agar (TSA, Difco Inc., Detroit, MI) and examined after 24, 48 and 72 h incubation at 37C and found to contain yeasts and molds. Initial storage trials ended after 8 days due to mold appearing on the meat slice surfaces. In order to eliminate the yeast/mold the meat slices were sterilized by irradiating in a ^{137}Cs gamma source (Lockheed Corp., Marietta, GA, dose rate 0.114 kGy/min). The dose rate was established using reference dosimeters (National Physical Laboratory, Middlesex, United Kingdom). The irradiated meat slices were aseptically dried using a circulating hot air drier (The National Drying Machinery Co., Philadelphia, PA) to specific water activities of 0.65, 0.70, 0.75, 0.80 or 0.82. The water activities of the samples were measured using an Aqua Lab CX-2 water activity system (Decagon Devices, Inc., Pullman, WA). The calibration of the meter was checked daily using known

salt standards. Each reported a_w value is an average of two measurements. The thickness of the meat slices were measured using a Vernier caliper (Monostat, KWB, Switzerland). The moisture content of the meat samples was obtained using a CEM AVC™-80 (Automatic Volatility Computer, CEM Corp., Matthews, NC). The dried meat was diluted 1:10 with 0.1% peptone water, blended for one minute at normal speed using a Stomacher 400 (Seward Medical, London, England) and filtered. The pH of the filtrate was monitored using a combination electrode (Orion Research, Inc., Boston, Ma) attached to an Orion Model 611 pH meter.

Cheese Samples

Pasteurized American cheese slices (8.7 $\pm$ 0.2 cm squares, 0.2 cm thick) were purchased from a local supermarket. The total background aerobic microbiota of the cheese slices were enumerated on both TSA and examined after 24, 48 and 72 h incubation at 37C and on Baird-Parker agar with EY-tellurite enrichment (BP, Difco) and examined after 24 and 48 h incubation at 37C. Since no colonies on either TSA or BP agars ($<$ 10 CFU/g) were observed, the cheese slices were not irradiated. The a_w, % moisture and pH were monitored throughout the study.

Water Activity Study

The transfer of moisture from cheese to meat was studied using sets of four sterile meat slices, a_w = 0.71, stacked to increase the sample thickness. The meat stack was placed in contact with a cheese slice of equal thickness so that only one meat slice was in contact with the cheese to simulate actual product. This meat slice was designated as slice 1. The stacks were vacuum packaged and stored at room temperature. Duplicate stacks were examined every 3 h for up to 12 h and then at 24 h. The meat and cheese slices were separated, and the a_w, sample thickness and % moisture were remeasured for each meat slice and cheese slice.

Challenge Study

Staphylococcus aureus 196E was obtained from the Eastern Regional Research Center (Philadelphia, PA) stock culture collection. The inoculum was prepared by culturing the organism for 18-24 h with shaking at 37C in brain heart infusion broth (Difco). The overnight culture was diluted with sterile 0.1% peptone water (Difco) to obtain a concentration of log 5-6 CFU/ml. A 10-fold dilution was made in sterile 25% NaCl (a_w = 0.83) in order not to add any moisture. This dilution was used immediately to inoculate samples (0.5 ml/50 g sample) to obtain an inoculum of log 2-3 CFU/g.

The primary study employed single slices of dried sterile meat (a_w = 0.70, 0.75 or 0.80) surface inoculated with the *S. aureus* and interfaced with a slice of pasteurized processed American cheese (a_w = 0.94). The thickness of the meat and cheese slices were equal (0.2 ± 0.05 cm). Aseptic techniques were followed throughout this study. The inoculated meat/cheese samples were placed in filter stomacher bags (SFB-0410; Spiral Biotechnology, Bethesda, MD) and inserted into plastic barrier bags with an oxygen transmission rate of 3.5 cc/100 in^2 in 24 h at 25C and 75% relative humidity (Koch, Kansas City, MO). The bags were vacuum heat sealed using a Multivac Model A300/16 gas packaging machine (W. Germany). Samples were stored at 19, 28 and 37C for up to 80 days and at a 6-h "square-wave" fluctuating temperature regime of 19-37-19C for up to 21 days. Each trial was replicated twice.

Duplicate samples were randomly chosen weekly from the stored products. The stomacher bag containing the meat/cheese sample was removed from the vacuum bag and peptone water (0.1%) added to obtain a 1:5 or 1:10 dilution. The entire contents were blended for one minute at normal speed using a Stomacher 400. The diluted samples were surface plated on BP agar using a Spiral Plater (Model D, Spiral Systems, Inc., Cincinnati, OH) and incubated for 24 h at 37C. The shiny black colonies surrounded by a clear zone were counted.

The growth of *S. aureus* within the meat portion was studied using the stacked meat (a_w = 0.72) cheese model described earlier. *S. aureus* was also inoculated between meat slice 1 (i.e., the slice in contact with the cheese) and slice 2. The stacks were vacuum packaged and incubated at 37C for up to two weeks. After the appropriate storage period meat slices 1 and 2 were removed from the stack and assayed for *S. aureus*. Duplicate samples were diluted with peptone water (0.1%) and surfaces plated on BP agar as described above.

RESULTS AND DISCUSSION

Meat and Cheese Preparation

The meat and cheese visibly deteriorated within 3 weeks of packaging. The cheese darkened (yellow-orange to orange color). The meat slices also darkened from a reddish brown to dark brown. The visual deterioration was accelerated at the higher temperatures.

The pH of the processed cheese was 5.7 ± 0.1 before being placed in contact with the inoculated meat slice and 5.5 ± 0.1 after 80 days of storage. The pH of the dried meat slices was 5.8 ± 0.2 before inoculation and remained constant for the duration of the studies. Thus, the pH of the meat and cheese in the current study was within the range that would be expected to support *S. aureus* growth (Tatini 1973).

Water Activity Study

The changes in a_w of stacked meat slices (initial a_w = 0.71) after being in contact with a cheese slice (initial a_w = 0.94) are shown in Fig. 1. The stacked meat slices were used to permit rapid layer separation for a_w measurement. The changes in a_w of the meat slices immediately adjacent to the cheese were rapid, with the a_w of meat slice 1 reaching 0.88 after 1.5 h of contact with the cheese. This increase in a_w was accompanied by an increase in moisture content from 20% to 25%. There was also an increase in the thickness of the first meat slice from 0.022 to 0.025 cm indicating moisture absorption (hydration). Changes in the a_w of the other slices were slower, and they had not fully equilibrated after 24 h.

Growth Studies

During the growth study with single slices of meat and cheese, water activity and % moisture changes occurred largely within the first 24 h, with the a_w of the meat slice increasing to 0.91 regardless of the initial a_w (Table 1).

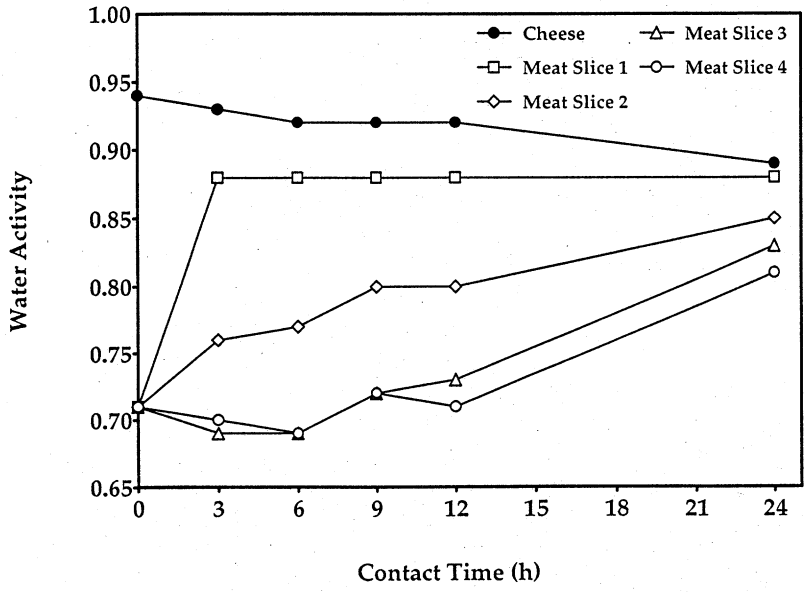

FIG. 1. CHANGES IN WATER ACTIVITY OF THE MEAT SLICES AND CHEESE WHEN STORED AT ROOM TEMPERATURE

TABLE 1.
WATER ACTIVITY AND % MOISTURE OF MEAT AND CHEESE SLICES FROM THE "SINGLE SLICE" GROWTH STUDIES

Temperature °C	Water activity				% Moisture			
	meat		cheese		meat		cheese	
	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
19	.80	.91	.93	.91	22.8	39.9	41.6	40.1
28	.80	.91	.93	.91	22.8	39.4	41.6	40.3
37	.80	.91	.93	.90	22.8	38.6	41.6	40.3
19	.75	.91	.92	.91	20.5	29.8	39.6	38.9
28	.75	.91	.92	.91	20.5	31.4	39.6	38.5
37	.75	.91	.92	.90	20.5	29.6	39.6	39.8
19	.70	.91	.93	.91	21.7	35.7	41.6	39.1
28	.70	.91	.93	.91	21.7	36.7	41.6	40.1
37	.70	.91	.93	.91	21.7	36.2	41.6	40.3

There was a corresponding decrease in the a_w and % moisture of the cheese. Subsequent sampling over the course of the storage period yielded values similar to the results for the 24 h samples, indicating no loss of moisture during storage. The estimated a_w value of 0.90 is above the reported minimum a_w for *S. aureus* growth under either aerobic or anaerobic conditions (Tatini 1973).

Representative growth profiles of *S. aureus* for the three incubation temperatures are illustrated in Fig. 2 a-c for meat slices with an initial a_w of 0.70, 0.75 and 0.80, respectively. Growth was apparent for all initial a_w for samples stored at 28 and 37C. A maximum population of 10^6 -10^7 CFU/g was generally achieved within 7 to 14 days of storage. Populations in the 37C samples declined with further storage, whereas those in the 28C samples remained constant. The decline in the 37C samples tended to occur at the same time as visual deterioration of product became evident. Growth in the 19C samples was more sporadic, occurring in only some of the samples. This could not be correlated with the initial a_w of the meat. *S. aureus* levels remained unchanged for extended periods in the 19C trials that did not support growth. Scheusner *et al.* 1973 and Smith *et al.* (1983) reported decreased growth of *S. aureus* at 19C. Kreisman and Labuza (1978) reported that *S. aureus* is capable of remaining viable over extended times at room temperature in processed cheese. When equivalent trials were performed using fluctuating incubation temperatures (19-37-19C) in conjunction with starting meat a_w of 0.65, 0.70, 0.75 and 0.82, the growth patterns observed were similar to those for isothermic incubation at 28 and 37C (Fig. 3).

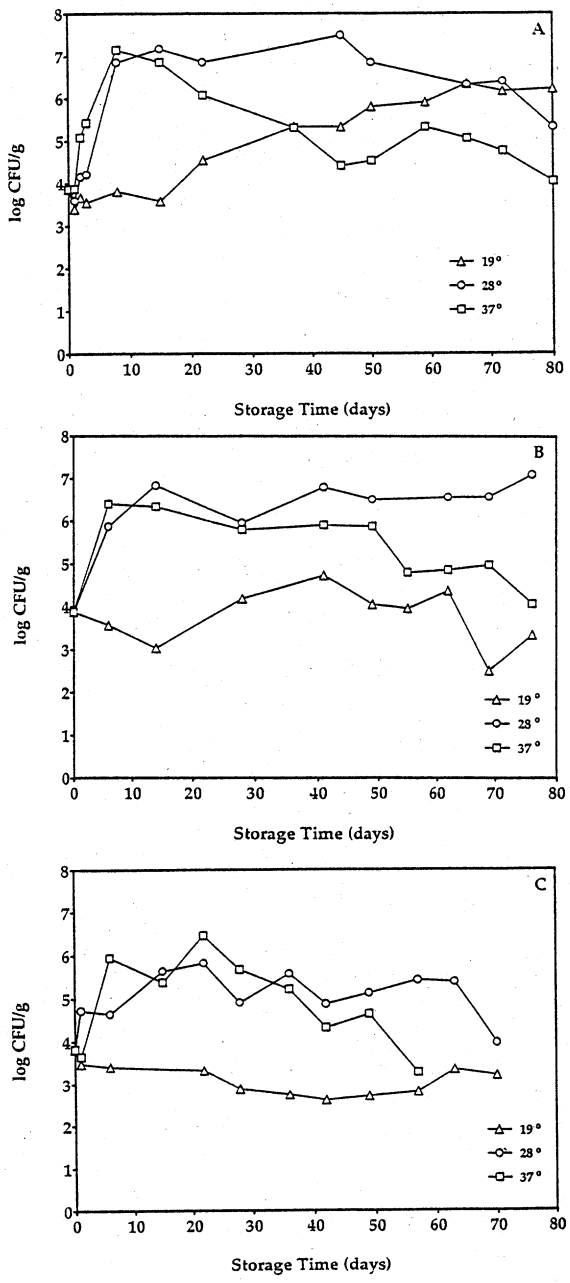

FIG. 2. GROWTH PROFILES OF *STAPHYLOCOCCUS AUREUS* AT THE MEAT-CHEESE INTERFACE AT 19, 28 AND 37C
The initial cheese's a_w is 0.94. A. meat's a_w = 0.70;
B. meat's a_w = 0.75;C. meat's a_w = 0.80.

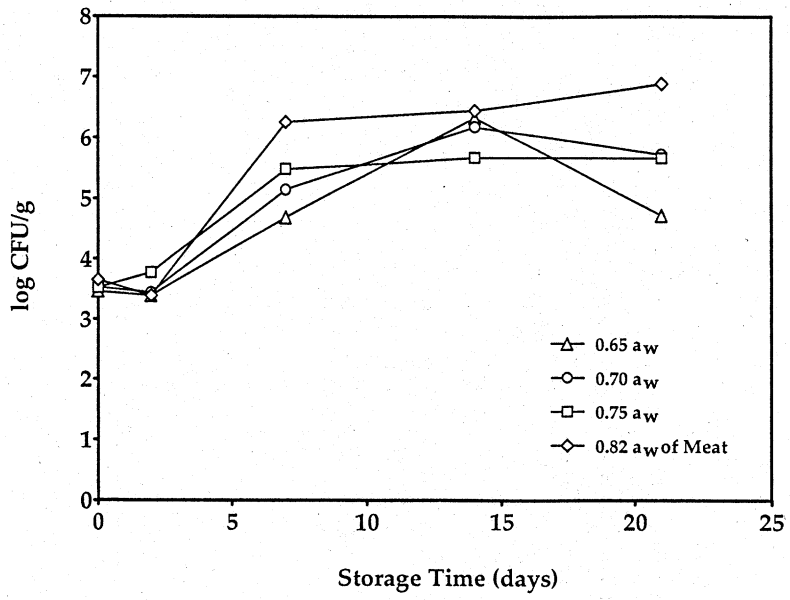

FIG. 3. GROWTH PROFILES OF *STAPHYLOCOCCUS AUREUS* AT MEAT-CHEESE INTERFACE AT FLUCTUATING TEMPERATURE (19-37-19C)

When *S. aureus* was inoculated between meat slice 1 and 2 (initial a_w = 0.72) and placed in contact with the cheese, about one log increase in cell number occurred after 2 weeks incubation at 37C (not shown). The a_w at the interface of slices 1 and 2 was estimated to be 0.86 after 1 week, with a % moisture increase of 7%. This indicates that the potential for, and rate of *S. aureus* growth is reduced the further the distance from the interface with the cheese, and is in agreement with the moisture transfer profile depicted in Fig. 1.

Under aerobic growth conditions *S. aureus* is reported to grow at a minimum a_w of 0.83 at 37C to 0.86 at 20C, and for anaerobic growth conditions the a_w range is 0.90 to 0.92 depending on incubation temperature (Bennett and Amos 1982; Lee *et al.* 1981; Kreisman and Labuza 1978; Tatini 1973; Untermann and Müller 1992)). In this study the estimated a_w at the meat/cheese interface was 0.90-0.91, which is well within the reported range for aerobic growth, and just within the reported a_w range for anaerobic growth.

Overall, the results of this study indicate that sufficient moisture can be transferred from the cheese portion of these products to the meat such that the water activities at the interface could support at least the transitory growth of *S. aureus*. This suggests that to be produced safely these products would either have to rigorously exclude halotolerant pathogens such as *S. aureus* during

manufacture and assembly, provide a secondary barrier such as acidification, or be refrigerated. Alternatively, the two components could be packaged in physically separated compartments to prevent moisture transfer.

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